

Comparison of the Tryptic Peptides of Wild Strains of Tobacco Mosaic Virus

Mutants of tobacco mosaic virus (TMV) derived under experimental conditions from the normal TMV strain *vulgare* show only minute differences, if any: one or only a few amino acids may be replaced by others. Besides these mutants a number of TMV-strains are known whose relationship to *vulgare* is not clear because they were found in nature. These strains show many biological differences when compared with the normal TMV strain; it is therefore interesting to study what chemical differences exist among them.

One of these wild TMV strains is *dahlemense*, isolated by Melchers (1) from tomato plants about twenty years ago. In contrast to the mutants derived from *vulgare*, which all produce local lesions on *Phaseolus vulgaris*, *dahlemense* does not give lesions on this plant. It shows green systemic symptoms on "Samsun tobacco" and produces local lesions on "Java tobacco". Its serological behavior is quite different from that of the *vulgare* strain or its mutants (2, 3). Figure 1 shows the electrophoretic mobility of whole virus and corresponding derived A protein of *vulgare*,

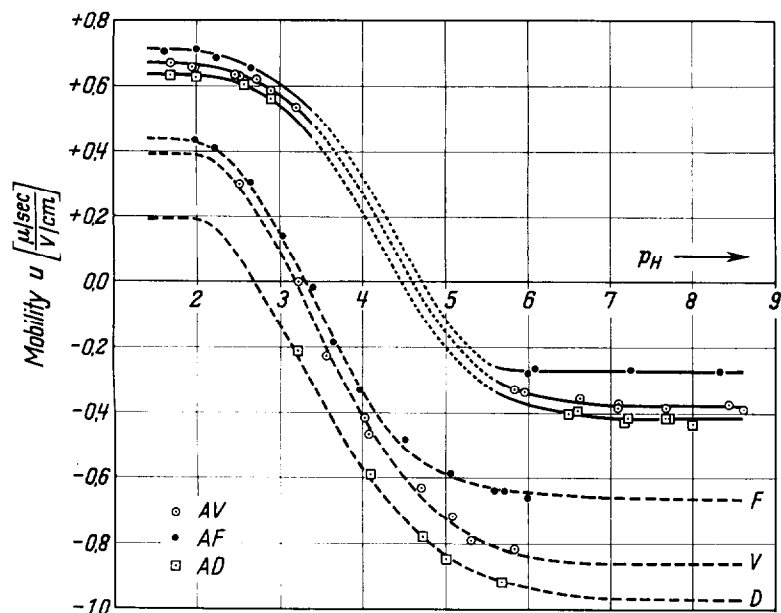


FIG. 1. Electrophoretic mobility of *vulgare* (V), *flavum* (F), and *dahlemense* (D) and their corresponding A proteins (AV, AF, and AD) (cf. (9)).

flavum, and *dahlemense*. From this it can be concluded that the number of acidic groups in *dahlemense* virus is higher than in *vulgare* and the number of basic groups lower. The isoelectric point of pH 2.7 is lower than that of any other mutant or strain of TMV. The amino acid composition has been previously studied by the DNP method (4), however, without splitting the protein into peptides.

In order to determine the exact amino acid composition of *dahlemense* its tryptic peptides were studied. The virus was split into ribonucleic acid and protein, the protein was digested with trypsin, and the tryptic

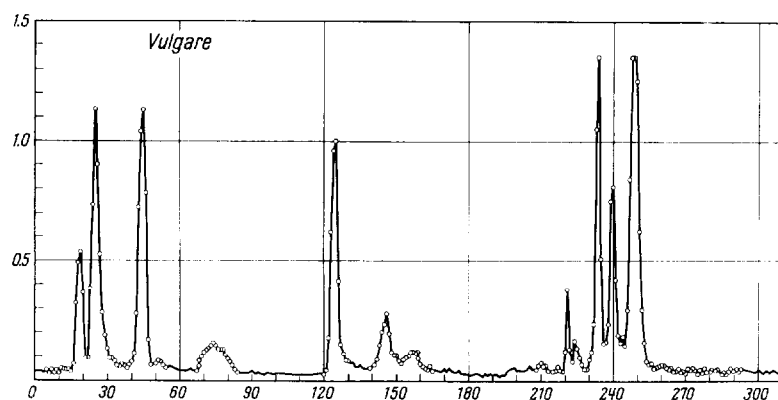


FIG. 2. Elution curves of the tryptic peptides of *vulgare*. Optical density after alkaline hydrolysis and ninhydrin reaction, plotted against number of fractions.

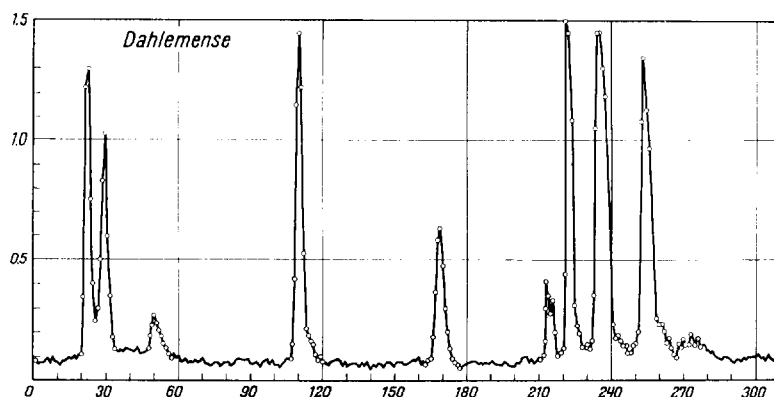


FIG. 3. Elution curves of the tryptic peptides of *dahlemense*. Optical density after alkaline hydrolysis and ninhydrin reaction, plotted against number of fractions.

	I		II		III		IV		V		VI		VII		VIII		IX		X		XI		XII		
	V	D	V	D	V	D	V	D	V	D	V	D	V	D	V	D	V	D	V	D	V	D	V	D	
Arg	1		1	1	1	1	1		1	1	2	2	1	1	1	1	-	-	-	-	1	1	1	1	
Lys	-		-	-	1	1	-		-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	
Asp	-		1	1	-	-	1		-	-	2	2	3	2	3	3	2	1	-	2	2	3	4	3	
Thr	1		-	-	1	3	1		-	-	1	1	4	4	2	3	-	-	2	3	-	-	4	3	
Ser	-		-	-	2	2	1		-	-	-	-	-	1	-	-	-	-	6	4	1	1	5	6	
Glu	1		-	-	3	5	-		-	-	-	-	4	5	-	-	-	-	1	2	1	1	6	6	
Pro	-		-	-	2	2	-		-	-	-	-	1	1	1	1	1	1	1	1	-	-	2	2	
Gly	-		-	-	-	-	2		-	-	-	-	-	-	1	1	-	1	1	2	3	-	-	1	1
Ala	-		-	-	-	-	-		-	-	2	2	3	2	3	2	-	-	1	2	1	1	4	2	
Val	2		-	-	3	3	-		1	1	2	2	1	1	2	1	-	1	1	1	1	2	1	3	
Met	-		-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	
Ileu	-		-	-	-	-	-		-	-	1	1	1	1	-	1	-	-	-	-	3	1	3	2	
Leu	-		-	-	-	-	-		-	-	-	-	1	1	4	4	-	-	1	2	2	2	4	4	
Tyr	-		-	-	-	-	1		1	1	-	-	-	-	1	1	-	1	-	1	-	-	1	1	
Phe	-		-	-	1	2	-		-	-	-	-	-	-	1	1	2	1	1	1	-	-	3	3	
Cys	-		-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
Try	-		-	-	1	1	-		-	-	-	-	-	-	-	-	-	-	1	1	-	-	1	1	
	5		2	2	15	20	7		3	3	10	10	19	19	19	19	7	7	17	24	12	12	41	41	

V = Vulgare, D = Dahlemense

V = *Vulgare*, D = *Dahlemense*

FIG. 4. Comparison of the tryptic peptides of *vulgare* and *dahlemense*.

peptides were separated by column chromatography; Figs. 2 and 3 show typical elution curves for *vulgare* and *dahlemense*. The peptides were purified if necessary by paper chromatography or high voltage electrophoresis and hydrolyzed, their amino acid composition was determined in an automatic amino acid analyzer. Details of the method have been described previously (5, 6).

Figure 4 shows the comparison of the tryptic peptides of *vulgare* and *dahlemense*. The composition of certain of the *dahlemense* peptides, e.g., No. IX with 7 amino acids, is very different from that of the corresponding *vulgare* peptides, whereas other peptides, e.g., VI with 10 amino acids, are exactly the same. It is interesting that *dahlemense* peptide X contains an amino acid, namely methionine, which is not present in *vulgare* or in any of its many mutants, but only in other very distantly related TMV strains, e.g., Holmes' ribgrass strain (7). In spite of the pronounced differences between the proteins of *vulgare* and *dahlemense* the number of amino acids within the protein chain of both strains is the same, namely 157.

The number of peptides obtained from *dahlemense* is 10, compared with 12 from *vulgare*; this is because two arginines, at which amino acid trypsin splits the chain, are exchanged for two other amino acids. *Dahlemense* peptide III with 20 amino acids corresponds to *vulgare* peptide III

with 15 plus peptide I with 5 amino acids. Similarly the C-terminal *dahlemense* peptide X with 24 amino acids (and no arginine and lysine) corresponds to the *vulgare* peptide IV with 7 plus peptide X with 17 amino acids. By comparing the tryptic peptides of several TMV strains and by determining their N-terminal amino acid residues, it has been possible to elucidate the arrangement of most of the tryptic peptides within the protein chain of TMV (8).

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REFERENCES

1. MELCHERS, G., *Naturwissenschaften* **30**, 48-49 (1942).
2. FRIEDRICH-FREKSA, H., MELCHERS, G., and SCHRAMM, G., *Biol. Zentr.* **65**, 187-222 (1946).
3. AACH, H. G., *Z. Naturforsch.* **12b**, 614-622 (1957).
4. AACH, H. G., *Z. Naturforsch.* **13b**, 425-433 (1958).
5. WITTMANN, H. G., and BRAUNITZER, G., *Virology* **9**, 726-728 (1959).
6. WITTMANN, H. G., *Z. Vererbungslehre* **90**, 463-475 (1959).
7. KNIGHT, C. A., *J. Biol. Chem.* **171**, 297-309 (1947).
8. WITTMANN, H. G., *Virology* **11**, 505-508 (1960).
9. KRAMER, E., and WITTMANN, H. G., *Z. Naturforsch.* **13b**, 30-33 (1958).

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